UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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JUN 13 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Trichlorfon (Mobay's Dipterix, Dylox and Negvon); EPA Nos. 3125-9, -7, -184 and 11556-32. Additional Data; EAB Deferal and RCB Deferals; and Label Change requests. (Trichlorfon Metabolite Questions).
Caswell #385
Tox. Br. Nos. 803787 and 80630.

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I. REQUESTED ACTIONS.

This Memorandum responds to the following data submissions; and deferrals to Toxicology Branch:

A) Toxicity Studies.

1) Rat Metabolism Study (Mobay Co. Report #945941; MRID #404381-01).
2) Mutagenicity Study (Mobay Co. Report # 94410; MRID #402772-01).

3) Rat Teratology Study (Mobay Co. Report #94638; MRID #402556-01.

4) Subchronic Neurotoxicity Study (Mobay Co. Report #94821; MRID #403512-00.

B) Deferrals to Toxicology Branch:

5) Residue Chemistry Deferral (Memorandum by Debbie Edwards, December 4, 1987), concerning the significance of TCF (Trichlorfon) metabolites found in wheat, potatoes, tomatoes, and goat tissues.

6) Environmental Assessment Branch Deferral (Memorandum by Allan Reiter and E. Regelman, April 8, 1988) concerning residues found in soil and aquatic studies; laboratory volatility, field dissipation and crop rotation studies.

C) Company Submissions:

7) Requested Label Changes (Mobay Company letter of August 27, 1987) to delete uses that might involve direct exposures to domestic animals.

8) Mobay's 6(a)(2) Submission (Mobay's letter of October 8, 1987) concerning high residues in a field trial study in California.

II. SUMMARY

The rat metabolism study was usable for an assessment of the kinetics of trichlorfon (TCF) however, the metabolites of TCF were not adequately identified. The rat metabolism study remains a data-gap.

The mutagenicity study was positive for sister chromatid exchange without activation but the effects were inconclusive in the presence of activation. Thus, a chromosome damage study remains a data-gap.

In the rat teratology study, there was no NOEL for fetal toxicity and there were questions about the choline esterase analyses. This study was classified Supplementary however, it was was not submitted to fill a data-gap as there are other acceptable teratology studies.

The subchronic neurotoxicity study was considered acceptable however, there was a discrepancy in the data that needs to be explained.

The metabolism study did not demonstrate the formation of DDVP from TCF. Information was cited by the Reviewer which indicates that either 1) no DDVP is formed from TCF or 2) only a very small and insignificant amount of DDVP may be formed from TCF. The question is not resolved at this time.

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A rereview of the many submitted metabolism studies and those in the literature should be made to assess the possibility that the DDVP formation is an artifact.

II. BACKGROUND

The Agency published a Rebuttable Presumption Against Registration (RPAR) notice in 1978 (FR 43 No. 77, April 20) based on studies that suggested that TCF may be oncogenic, teratogenic, fetotoxic and mutagenic but withdrew the RPAR because the existing data was not adequate for a valid risk assessment.

The Agency also completed a Registration Standard (Guidance for the Reregistration of Pesticide Products Containing Trichlorfon as the Active Ingredient) on June 30, 1984. The Toxicology Branch Chapter (Irving Mauer, March 13, 1984) listed a number of data gaps which included a general metabolism study and a chromosome aberration study (see Section E, page 46 from the Chapter, Appendix 1). A number of other studies have been submitted to fill other data gaps.

A number of investigations cited in the literature have concluded that the metabolites and/or conversion products of of TCF can form DDVP, a volatile toxic insecticide which has been shown to possess oncogenic capacities (DDVP Guidance package, September 1987). However, many metabolic investigations concluded that 1) DDVP was not a metabolite of TCF; 2) that DDVP was formed only to a limited; or 3) DDVP may be formed as an artifact of the analytical method. One report showed DDVP as a contaminant of technical TCF. The oncogenic virulence of DDVP has not been finally established (Class B2 or C oncogen?).

III. REVIEW OF SUBMISSION AND DEFERRALS.

A. Rat Metabolism Study.

H.S. Shaw, II, et al: Excretion and Metabolism of Dylox in Rats. Mobay Report No 94594, June 30, 1987.

The DER by S.B. Gross for this study is included with this memorandum as Appendix A. In summary, radiolabeled TCF was administered to rats by gavage and followed as it was excreted in the urine, feces and expired air over a 4 day period. The levels of radioactivity in the tissues were measured at the end of the 4 day period. Approximately 90% of the radioactivity was excreted within 24 hour with 50% in the urine, 20% in the feces and 20% in expired air. About 2% of the radioactivity was found in the tissues. The investigators separated metabolites in the urine and feces but failed to identify the metabolites. Therefore, their claim that TCF was converted to DDVP was unsubstantiated. The study has therefore been labeled as SUPPLEMENTARY because of the failure to identify any of the metabolites. The data-gap for an adequate metabolism study still persists.

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B. Mutagenicity Study.

Donald L. Putman: Sister Chromatid Exchange (SCE) Assay in Chinese Hamster Ovary (CHO) Cells performed by Microbiological Associates, Bethesda, MD, 7/15/86. Mobay Report No. 94410.

The DER for this study was done by Irvin Mauer of Toxicology Branch and is included here as Appendix B. The study was considered ACCEPTABLE in the absence of activation and showed a positive for induction of SCE at 50 and 100 ug/mL with evidence for a dose response. INCONCLUSIVE in the presence of S9 activation, providing only presumptively positive induction of SCE at 75, 150 and 300 ug/mL, with minimal dose-response, thus requiring a confirmatory assay at tighter-spaced dose levels. Therefore, the data-gap for chromosomal aberations has only been partially filled.

C. Rat Teratogenicity

Kowalski, R. L., et al: A teratology study with Dylox\ technical (Trichlorfon) in the rat. Carried out by Miles Laboratories, Elkhart, IN, June 22, 1987. Mobay Report No. 94638.

The DER for this study was performed by Dynamac contract No. 337-A by Guillermo Millicovsky and is included here in Appendix D. In summary, pregnant rats were given TCF in food at levels of 500, 1125 and 2500 ppm on days 6 through 15 of gestation. None of the treated animals showed clinical signs of toxicity. The choline esterase levels were depressed in all treatment groups. However, variability of the esterase determinations was so variable that it was not possible to determine the biological significance of the choline esterase effects. There were incomplete ossifications of the skulls, ribs, cervical arches, thoracic cetnra and sternbrae of fetus in all treatement groups following a dose-response pattern with only those pups in the high dose group achieving statistical significance. The LOELs for maternal and developmental toxicity was considered to be 500 ppm (LDT group) and the study was considered to be SUPPLEMENTARY due to the absense of a NOEL based on skeletal growth inhibition seen as dose related in all treatment groups and the high variability of the choline esterease levels.

The Agency has an acceptable rat teratology (based on the one-liner listings), therefore it is not necessary for this study to be repeated.

D. Subchronic Neurotoxicity Study in Rats.

R.H. Hayes and W.W. Raam: Subchronic Delayed Neurotoxicity Study of Trichloroform Technical (Dylox\) with Hens. Mobay Corporation Report No. 94821, August 28, 1987.

The DER for the study was done by William Greear of the Toxicology Branch and is included here in Appendix C. In summary, TCF in corn oil/ acetone (90:10) was administered by gavage to hens at levels of 3, 9, and 18 mg/kg daily for 13 weeks. There was no overt clinical indications of a response characteristic of delayed neurotoxicity; however, there was a slight histological effect on nervous tissue manifested as axonal degeneration present in hens of the 18 mg/kg test group. The NOEL was 9 mg/kg and the LEL 18 mg/kg based on the slight axonal degeneration.

The study was considered CORE-GUIDELINE (tentatively) based on need for the Sponsor to explain the discrepancy in reporting results for the forced activity (TURF) test in which results were provided for Hens Nos. 5 and 9, when the hens were given 4-digit numbers. If this discrepancy is resolved, this study will serve to fill the data gap for a subchronic neurotoxicity study.

IV. METABOLITES OF TRICHLORFON

The following abbreviations are used in this section: DCA = dichloroacetic acid; DCE = dichloroethanol; TCE = trichloroethonol.

A. Residue Chemistry Deferral.

Memorandum by Debra Edwards: Followup to the Trichlorfon Registration Standard -- Plant and Ruminant Metabolism (RD Record No. 204303, RCB No. 2837, MRID Nos. 4033860-1, -3, -4, -5). December 4, 1987.

In tomatoes, TCF was the key residue (greater than 60% up to 10 days after application. DDVP which was found early on (13% at 2.5 hours disappeared by day 7 and was replaced by DCA (41%) and DCE (30%) by day 21. There was an erratic level of TCE over the 21 day period. A measureable portion of the radioactivity was incorporated into glucose, increasing to 7% by day 21.

In potatoes, about 88% of the radioactivity was converted to glucose, 7-8% as DCE and the rest (4-5%) insoluble (unidentified).

In wheat, TCF accounted for approximately 15% of the residues, while DCA accounted for 30-40%.

In the goat metabolism study, identified residues ranged from 32 to 86% of the administered TCA as found in liver, kidney, muscle, fat and milk. Glucose was the major residue (64%) in milk while DCA (22%) was the only other metabolite in milk. DCA glucuronide (a water soluble metabolite) was the only major (65%) metabolite in fat. DCA conjugates were major metabolites in muscle and kidney while liver had a general distribution of several metabolites, somewhat evenly distributed. DDVP was not found as such and only a small amount of desmethyl DDVP (1% or less) in liver and kidney.

Conclusions. The submitted studies for wheat, potatoes and tomatoes indicated that TCF undergoes dehydrochlorination and rearrangement to form DDVP, and further conversion to dichloracetic acid (DCA), dichloroethanol (DCE), dichloroacetaldehyde (DCAd) and trichloroethanol (TCE). The analyses for used to determine a number of these metabolites involved acid hydroysis which may have converted (see below) TCF to DDVP/metabolites. Therefore this Reviewer recommends further assessment of the methods for possible actifactual conversion of TCF to DDVP/metabolites (Conversation of 5/10/88 with Debra Edwards).

B. Exposure Assessment Branch Deferral.

Memorandum of April 8, 1988 by Allan Reiter through E. Regelman summarizing the Dynamac Corporation Report Trichlorfon Addendum, Feb. 12, 1988 concerning the following studies:

1) Anaerobic soil metabolism and aquatic metabolism.
2) Laboratory Volatility.
3) Terrestrial field dissipation
4) Accumulation in confined rotation crops.

Observations of these studies taken from Allan Reiter's review include:

Anaerobic Soil Study. Soils treated with radiolabeled TCF were analyzed after 35 days post treatment. Analysis of the treatment solution immediately prior to the treatment indicated the degradate DDVP was present at 13%. Only 85% of the label was accounted for after 13 days and 54-79% after 35 days. The study was considered UNACCEPTABLE.

Laboratory Volatility. Radiolabeled TCF formulated as an 80% soluble powder was applied to sand soil and the radioactivity followed for 14 days. 1% of the applied activity was trapped in methanol, 13% in aqueous NaOH and 84% remained in the soil. DDVP itself was used to test the ability of the system to trap volatile constituents, however TCF apparantly was not especially volatile. The study was considered ACCEPTABLE and tends to support the notion that TCF was not converted to DDVP which does volatilize.

Dissipation in Field Soil. Radiolabeled TCF was applied to field soil and the activity in the soil followed for 32 days. Although TCF residues were detected, the registrant concluded there were no toxic metabolites (of DDVP) formed; however, there were no recovery or validity data on the methods provided.

Accumulation Studies on Rotational Crops. EAB concluded that the study was inadequate because the actual immediate post treatment concentration in soil and plants was inadequate, recovery values for platn samples following extraction were

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not reported and soil analysis data were not provided for all planting and harvesting intervals.

Conclusions. These studies did not adequately characterize the TCF conversion products nor did they demonstrate any conversions of TCF to DDVP/metabolites. Toxicology Branch cannot comment on the significance of DDVP/metabolites until the amount and type is more adequately ellucidated (discussion with Allen Reiter and Emil Regelman, 5/11/88).

C. Metabolism of Trichlorfon to DDVP.

Metabolic Profiles. Figure 1 taken from page 31 of the Mobay rat metabolism study (Shaw et al, 1988) lists a number of assumed metabolites derived of TCF (on the left of the figure) and those derived from DDVP (on the right side of the figure). The world literature indicates a number of metabolic schemes for TCF and DDVP (see references cite below). The ellucidation of metabolic conversions for both DDVP and TCF do not come from any one study or any one biological system but are derived from a variety of different studies using different biological media. The procession from one metabolite to another in the apparently orderly fashion is usually derived from different studies using different species: plants, animals, microorganism, soil, etc. Discrepancies result from different studies due to the experimental designs, analytical methods, etc.

The metabolic breakdown of TCF included here taken from page 41 of the CEC (1977) monograph shows a metabolic profile which includes the conversion of TCF to DDVP and its metabolites. These authors, however, indicate in the text of the monograph (page 40) that the conversion of TCF to DDVP in mammals is very limited (citing a 1966 reference).

Figure 7.4 (taken from page 346 of Hayes, 1982 b) present one schematic for the metabolism of DDVP in animals. According to Hayes, the initial metabolites are o,o- dimethyl phosphae and o-methyl-o-2,2 dichlorovinyl phosphate (desmethyl-dichlorvos). The breakdown of the compounds shown in Figure 7.4 are found in the intestinal lumen of intact animals; however, only dichlorethanol (DCE) has been found in blood and none in tissues. Conjugated DCE is found in the urine. Metabolism of DDVP is considered remarkably rapid. If one is to assume that DDVP is formed from TCF, DCE and its conjugates should be found in blood, urine and tissues of animals given TCF in metabolism studies.

Questions Concerning the Conversion of TCF to DDVP. Many of the reports in the literature (cited by Bull and Ridgeway (1969) lists a number of studies which do not indicate or did not find DDVP as a metabolite of TCF. Based on information presented above plus the following observations there is reason to doubt the formation of DDVP from TCF in mammals:

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Pages 8-10 - *Access to FIFRA health and safety data is restricted under FIFRA section 10(g)*

1) Lack of DDVP/Metabolites. Several studies have not identified DDVP/metabolites in TCF metabolic studies. This may be because:

a) DDVP/metabolites were not formed.
b) The levels of DDVP/metabolites were below detectable limits used in the studies.

2) Artifactial Formation of DDVP?

a) Barthel et al. (1955) showed that TCF is converted to DDVP under acid or alkaline conditions in non-biological aqueous media. Therefore it is possible that DDVP was formed during acid hydrolysis or alkaline treatment as part of the analytical methods used. Current studies need to be reevaluated for this possibility.

b) Based on biotransformation of other phosphate esters, it does not seem likely to this reviewer that TCF should convert to DDVP in vivo. Instead, one would expect TCF to hydrolize to methyl phosphates and TCE conjugates.

3) Contamination? WHO (1972) indicated (page 184) that technical TCF contained DDVP (2000 ppm) and the DDVP metabolite, DCAd (300 ppm) as contaminants. This raises a question as to whether TCF used in some of the studies reported in the literature were contaminated.

4) Toxicity Comparisons. The acute oral toxicity (LD50) of of DDVP in rats is about 60 mg/kg while the toxicity of TCF is about 600 mg/kg, about 1/10th as toxic. Both materials are considered to be rapidly metabolized. This suggests that any formation of DDVP from TCF is very small or non-existant.

5) Cancer considerations. We are concerned about the DDVP metabolites from TCF because of the possible carcinogenicity considerations and possible special review. Levels of DDVP/metabolites in mammals and in plant products seem to be formed at low concentration, when they appear in the materials being analyzed. The WHO (1972), pages 193-94, discussion on TCF cites a number of studies which suggested that TCF was tumorigenic in its own right. These studies, and other studies to be submitted by the registrant, should be evaluated before considering TCF for special review.

V. COMPANY REQUESTS

A. Requested Label Changes.

Mobay Corporation requested in its letter of August 27, 1987 from John Thornton changes in several labels which are intended to remove the requirement for §85-2 Domestic Animal Safety data. The company interprets this as relating to end-use products which provide exposure through direct application

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for pest control. Accordingly, they asked for changes for the following labels:

Dipterex Technical Insecticide (EPA #3125-9) to be used only for the manufacture of insecticides.

Diperex Sugar Bait Insecticide (EPA #3125-7).

Neguvon Brand of TCF Cattle Insecticide Pour-on (EPA 11556-32. The company is deleting the use on dairy cattle of breeding age; however, it is still to be used on other cattle??

Dylox 80% Soluble Powder Crop Insecticide. (EPA #3125-184). A re-entry interval of 24 hours is set for field crops. This in established based on re-entry data??

Recommendations. Toxicology Branch has no objections to the label changes as they relate to domestic animal applications except for Neguvon Pour-on. This label allows for direct application to domestic animals and therefore this formulation should be tested as required for Domestic Animal Safety 85-2. Other questions are noted above.

B. 6(a)(2) Data Submission.

John Thornton of Mobay Corporation indicated in his October 9, 1987 letter to William Miller (RD) that some TCF residues analyses of cotton conducted in California were above established tolerance levels. The company indicated that these tests are aberrant and that they will carryout additional testing on the locations used. Toxicology Branch notes that the information provided in the Mobay letter is inadequate to determine the extent that the residues might be a problem, especally when the Agency has been in the process of trying to obtain information from the registrant in order to determine acceptable residue levels through the Data Call-in process.

VI. REFERENCES

Barthel, W.F. et al. (1955). J. Am. Chem. Soc. 77: 2424.

Bull, D.L., and Ridgway, R.L. (1969). Metabolism of trichlorfon in animals and plants. J. Agr. Food Chem. 17: 837-841,

CEC (1977) "Organophosphorus Pesticides. Criteria (Dose/Effect Relationships)d for Organophosphorus Pesticides." Commission of the European Comunities, Pergamon Press. NY.

FAO/WHO (1978). Trichlorfon. Pages 239 to 255 in "Pesticide Residues in Food- 1978". Food and Agriculture Organization of the United Nations, Rome.

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Hayes, W. (1982a). Trichlorfon. Pages 351-356 in "Pesticides Studied in Man". Williams and Wilkins, Baltimore.

Hayes, W. (1982b). Dichlorvos. Pages 343-351 in "Pesticides Studied in Man". William and Wilkins, Baltimore

WHO (1972). Trichlorfon. Pages 183-230 in WHO Pesticide Series, No. 1. 1971 Evaluations of Some Pesticides in Food. World Health Organization.

WHO (1976). Trichlorfon. Pages 379 to 392 in WHO Pesticide Series, No. 5. 1975 Evaluations of Some Pesticide Residues in Food. World Health Organization, Geneva.

Appendix 1

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[E] DATA GAPS/ISSUES OF CONCERN

EPA does not have adequate studies to satisfy the following generic data requirements for TGAI trichlorfon according to CFR Section 158.135 Toxicology:

81-3 - Inhalation LC_{50} - Rat - N.

82-1 - 90-Day Feeding - Rodent, Non-Rodent (mod? — low signif?)

82-2 - 21-Day Dermal

82-3 - 90-Day Dermal (depending on results of 21-day dermal) (repeated?)

82-4 - 90-Day Inhalation - Rat

82-5 - 90-Day Neurotoxicity

83-1 - Chronic Toxicity - Rodent and Non-Rodent (monkey. Guideline 83-1)

83-2 - Oncogenicity - Rat and Mouse

84-2 - Mutagenicity: Chromosome Aberrations

85-1 - General Metabolism (using the PAI or PAIRA)

Product specific data requirements for MUP's must also be satisfied in the following categories:

81-3 - Inhalation LC_{50} - Rat

81-5 - Primary Dermal ~~Inhalation~~ Irritation

81-6 - Dermal Sensitization

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EPA: 68-02-4225
DYNAMAC No. 337-A
April 21, 1988

DATA EVALUATION RECORD

TRICHLORFON

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Kowalski, R. L., Clemens, G. R., Bare, J. J., and Hartnagel, R. E. A teratology study with DYLOX® technical (Trichlorfon) in the rat. (Unpublished report No. 94638 by the Corporate Toxicology Department, Miles Laboratories, Inc., Elkhart, IN, for Mobay Corporation, Stilwell, KS; dated June 22, 1987.) Accession No. 402556-01.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 4/22/88

1. CHEMICAL: Trichlorfon, DYLOX® technical, dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate.

2. TEST MATERIAL: Technical grade trichlorfon, batch No. EHR-150-8-79, was described as a water soluble white crystalline solid containing 99% active ingredient.

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: Kowalski, R. L., Clemens, G. R., Bare, J. J., and Hartnagel, R. E. A teratology study with DYLOX® technical (Trichlorfon) in the rat. (Unpublished report No. 94638 by the Corporate Toxicology Department, Miles Laboratories, Inc., Elkhart, IN, for Mobay Corporation, Stilwell, KS; dated June 22, 1987.) Accession No. 402556-01.

5. REVIEWED BY:

Guillermo Millicovsky, Ph.D.
Principal Reviewer
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Signature: G Millicovsky
Date: 4/20/88

Claire Kruger-McDermott, Ph.D.
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6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenicity and Reproductive Effects
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Signature: By direction of I. Cecil Felkner
Date: 4/20/88

Stanley Gross, Ph.D.
EPA Reviewer

Signature: Stanley Gross
Date: 5/24/88

Albin Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 6/7/88

7. CONCLUSIONS:

A. We assess that the LOELs for maternal and developmental toxicity were both 500 ppm, the lowest dose tested, based on effects noted at all levels in this study (see Section 14 for details). The NOEL for this study was not established.

B. This study is classified CORE Supplementary due to the absence of a NOEL, and due to the variation in the reported values for cholinesterase inhibition.

8. RECOMMENDATIONS:

We suggest that the registrant submit for review a description of the methods used for determination of cholinesterase activity.

9. BACKGROUND:

Dose level selection for this study was based on a range-finding study conducted on groups of eight pregnant rats fed diets containing 500, 1000, 1500, 2000, 2500, 3000, 3500, or 4000 ppm trichlorfon. Reductions in food consumption and body weight were noted at dietary levels of 1500 and 2000 ppm or greater, respectively. Reductions in maternal plasma cholinesterase activity were noted at all levels tested. Increased resorptions occurred in animals fed 3000 or 4000 ppm; increased postimplantation loss was reported at doses of 3000 ppm. No malformations were reported for any group.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: See Appendix A of this DER for details.

Trichlorfon was mixed once with Purina Certified Rodent Chow #5002 at concentrations of 0 (control), 500, 1125, and 2500 ppm (w/w); these diets were frozen until needed. Each day, an aliquot of each of the above diet preparations was removed from the freezer and made available ad libitum. Diets were analyzed prior to study initiation to determine the concentration, homogeneity, and stability of the test material.

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[1]Only items appropriate to this DER have been included.

Naive, sexually mature Charles River Crl:CD® BR male and female rats were obtained at 11 weeks of age. After an acclimatization period of at least 7 days, two females were paired overnight with one male. Mating was determined by the presence of spermatozoa in vaginal smears. The day of mating was designated gestation day (GD) 0. A total of 132 inseminated females were randomly assigned to four groups consisting of 33 animals each. Five animals per group were killed on GD 16, and the remaining animals were killed on GD 20.

Animals were individually housed in a room maintained at 65-76°F, 40-65% relative humidity, and 12:12 hour light:dark photoperiod. Test diets were presented to the animals on GD 6-15. Calculated test material intake was approximately 45, 102, and 227 mg/kg/day for the 500-, 1125-, and 2500-ppm groups, respectively.

Maternal body weights were recorded on GD 0, 6, 8, 10, 12, 15, and 20; food consumption was recorded on GD 0, 3, 6, 8, 10, 12, 15, and 19. Animals were observed daily for overt signs of reaction to the treatments.

Five females from each group were killed on GD 16 by CO_2 inhalation; these animals were used to assess brain, erythrocyte, and plasma cholinesterase activity. The animals were necropsied, and their pregnancies were confirmed. The remaining females were killed on GD 20 by CO_2 inhalation, and the cholinesterase activity of erythrocytes, plasma, and brain tissue was assessed for the first 20 pregnant females per group. The methods used to assess cholinesterase activity were not described.

Laparotomies and necropsies were conducted on all females killed on GD 20; their corpora lutea were counted and recorded, and the weights of the gravid uteri were determined. Fetuses and resorptions were removed from the uterine cavities; fetal viability and sex, and fetal and placental weight were determined. All fetuses were externally examined. The thoracic and abdominal cavities of approximately half of the fetuses from each litter were examined fresh. Brains from 20 fetuses per group were removed, examined grossly, frozen, and used to determine fetal brain cholinesterase activity. Following the visceral examinations, these fetuses were fixed in Bouin's solution, and their heads were sectioned and examined.

The remaining fetuses were fixed in alcohol, eviscerated, stained in Alizarin Red-S, and examined for skeletal abnormalities.

Data were statistically analyzed; a summary of statistical analyses is included in Appendix L of the study report.

12. REPORTED RESULTS:

A. Test Diets: Reported analytical data indicated that under the conditions of this study, the concentration, homogeneity, and stability of the test material in the test diets were acceptable.

B. Maternal Effects: No overt clinical signs of toxicity were reported for any group. Maternal body weight gains and food consumption were slightly (but significantly) reduced for the group fed 2500 ppm when compared with control values (Tables 1 and 2). Weight gains and food consumption for the other groups were comparable to those of controls. Analyses of cholinesterase activity in plasma, erythrocyte, and brain tissue of females killed on GD 16 suggest that significant enzyme inhibition resulted at all levels tested (Table 3); on GD 20, however, plasma and erythrocyte cholinesterase values were returning to normal, but brain cholinesterase inhibition was significant at all dosage levels. The authors assessed that values showing inhibitions greater than 20% were toxicologically relevant.

No adverse effects associated with the test material were noted at necropsy or in the reproductive parameters (Table 4) evaluated.

C. Developmental Effects: No adverse effects were noted in litter size or fetal body weight at any dosage level (Table 4). Fetal viability and sex ratios were comparable for all groups. No toxicologically meaningful reductions in brain cholinesterase activity were noted for fetuses recovered on GD 20 (Table 3).

External and visceral examinations did not suggest adverse effects at any dose level; however, statistically significant increased incidences of skeletal findings were observed among fetuses from mothers fed 2500 ppm (Table 5).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that the test material was toxic to pregnant dams at all levels tested. Developmental toxicity occurred at 2500 ppm (based on skeletal effects); 1125 ppm was the developmental NOEL.

B. A quality assurance statement was signed and dated on June 22, 1987.

TABLE 1. Effects of Trichlorfon on Maternal Body Weights in Rats

	Dosage (ppm)			
	0	500	1125	2500
Number of Females	22	25	24	24
GD 0	231.0±1.8[a]	235.3±2.6	231.8±2.2	235.7±2.6
GD 6	251.3±2.5	254.9±3.0	254.9±2.7	256.6±2.9
GD 15	300.6±3.0	303.8±4.0	306.3±3.1	304.8±4.2
GD 20	367.6±5.5	376.8±5.5	376.4±4.0	372.7±6.1
% Gain GD 6-15	19.6	19.2	20.2	18.8
% Gain GD 0-20	59.1	60.1	62.4	58.2
% Corrected Gain[b]	29.9	27.1	27.6	24.1**

[a]Values represent group mean (g) ± SE.

[b]Values obtained by subtracting gravid uterine weight from GD 20 body weight.

**Significantly different from control value ($p \leq 0.01$).

TABLE 2. Effects of Trichlorfon on Maternal Food Consumption in Rats

	Dosage (ppm)			
	0	500	1125	2500
Number of Females	22	25	24	24
GD 1	20.5±1.1[a]	21.3±0.6	20.9±0.6	20.2±0.8
GD 7	22.4±0.7	21.8±0.6	21.4±0.6	19.0±0.6*
GD 9	23.6±0.5	22.4±0.6	22.3±0.4	21.3±0.6*
GD 16	26.3±0.6	27.8±0.6	27.3±0.5	25.5±0.7
GD 20	27.4±0.5	26.1±0.6	26.9±0.7	27.0±0.7

[a]Values represent group mean (g/rat/day) ± SE.

*Significantly different from control value ($p \leq 0.05$).

TABLE 3. Effects of Trichlorfon on Maternal and Fetal Cholinesterase Inhibition in Rats

	Dosage (ppm)			
	0	500	1125	2500
Number of Females GD 16	5	4	4	4
Plasma	0	43.2*,[a]	55.9*	58.7*
Erythrocyte	0	23.5	33.8	36.3
Brain	0	15.4*	29.6*	49.0*
Number of Females GD 20	20	20	20	20
Plasma	0	9.9	4.3	15.1
Erythrocyte	0	11.2	3.6	15.1
Brain	0	12.1*	21.3*	44.0*
Number of Fetuses	20	20	20	20
Fetal brain	0	32.6	16.2	19.5

[a]Values represent percent reduction from control value.

*Significantly different from control value ($p \leq 0.05$).

TABLE 4. Summary of Reproductive Data from Rats Exposed to Trichlorfon

	Dosage (ppm)			
	0	500	1125	2500
Number of Females	33	33	33	33
No. (%) Pregnant	27(81.8)	29(87.9)	28(84.8)	29(87.9)
No. Aborted	0	0	0	0
No. Corpora Lutea[a]	14.6	16.0	15.5	15.8
No. Implantations[a]	12.4	14.9	15.4	14.8
No. Resorptions[a]	0.7	0.9	1.0	0.9
No. Females With More Than One Resorption	2	5	7	6
% Preimplantation Loss[a]	15.7	6.8	3.9	7.7
% Postimplantation Loss[a]	6.8	5.8	6.9	5.6
Litter Size[a]	11.7	14.0	14.4	13.9
Fetal Body Weight[a]	3.6	3.5*	3.6	3.7

[a]Values represent group means.

*Significantly different from control value ($p \leq 0.05$).

TABLE 5. Summary of Fetal Findings from Rats Exposed to Trichlorfon

	Dosage (ppm)			
	0	500	1125	2500
Number of Fetuses Examined	133	181	178	172
Skull (%)				
Incompletely ossified	45(33.8)	85(47.0)	86(48.3)*	101(58.7)**
Sutures enlarged	9(6.8)	14(7.7)	14(7.9)	39(22.7)**
Fontanelle enlarged	10(7.5)	22(12.2)	22(12.4)	47(27.3)**
Ribs (%)				
Incomplete ossified	0	0	0	14(8.1)**
Wavy or curved	2(1.5)	1(0.6)	0	34(19.8)**
Cervical Arches (%)				
Incompletely ossified	70(52.6)	101(55.8)	112(62.9)	115(66.9)*
Thoracic Centra (%)				
Incompletely ossified	58(43.6)	81(44.8)	91(51.1)	114(66.3)**
Sternebrae (%)				
4th incompletely ossified	57(42.9)	93(51.4)	85(47.8)	107(62.6)**
5th unossified	28(21.1)	38(21.0)	50(28.1)	72(42.1)**

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Based on the data presented in the study report, we assessed that the test material produced toxicologically significant inhibitions of maternal cholinesterase activity at all levels tested. Cholinesterase values for animals within each group showed considerable variability; however, we were unable to establish the validity of the methodology used since the authors did not present the methods for cholinesterase activity measurement. Slight reductions in corrected body weight and food consumption were noted at 2500 ppm. No adverse effects in clinical signs, necropsy findings, or reproductive data were noted for any dosage level.

Results from fetal brain cholinesterase activity were inconclusive (Table 3); a dose response was not present, and as we stated earlier, we were not able to determine the validity of the methods used by the testing laboratory. The only adverse developmental effects noted were dose-related increases in the incidence of ossification reductions in fetal skulls, vertebrae, and sternebrae noted at all dosage levels; compound-related effects on incompletely ossified ribs were noted only at 2500 ppm. As shown in Table 5 of this review, the above skeletal effects reached statistical significance at 2500 ppm; significant increases in the incidence of incomplete skull ossification occurred at 1125 and 2500 ppm. In addition, a significant increase in malformed (wavy or curved) ribs occurred at 2500 ppm. Based on the above findings, we assess that slight increases in skeletal effects were produced at the lowest dosage tested and that most of these effects increased in a dosage-related manner, reaching statistical significance at the high dosage level.

B. The only major difference between the conclusions reported by the study authors and those of the reviewers is related to the developmental NOEL. We concur with the author that statistically significant adverse effects in skeletal development occurred at 2500 ppm; however, the percent of affected fetuses increased with dosage, and we therefore assess that the slight (nonsignificant) increases noted at 500 and 1125 ppm (compared with controls) were also manifestations of compound toxicity.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods.

APPENDIX A

Materials and Methods

26

Pages 27-34 - *Access to FIFRA health and safety data is restricted under FIFRA section 10(g)*

006745

~~006805~~

TOXICOLOGY BRANCH: DATA REVIEW

Reviewed By: Irving Mauer, Ph.D. TB Project: 8-0378A
Toxicology Branch
Hazard Evaluation Division Date: 03-15-88

Through: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division

Chemical: Trichlorfon Caswell: 385
EPA Chem: 057901

Study Type: Mutagenicity - DNA Damage - Repair In Vitro (CHO/SCE)

Citation: Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells

Accession No.: 402772-01

MRID No.: N/A

Sponsor: Mobay Corporation
Division of Bayer USA
Kansas City, MO

Testing Lab: Microbiological Associates (MA)
Bethesda, MD

Study No.: MBA-T4491.334 (Mobay Report No. 94410)

Study Date: July 15, 1986

TB Conclusions/Evaluation:

ACCEPTABLE in the absence of activation; positive for induction of SCE at 50 and 100 ug/mL with evidence for a dose response. INCONCLUSIVE in the presence of S9 activation, providing presumptively positive induction of SCE at 75, 150, and 300 ug/mL, with minimal dose-response, thus requiring a confirmatory assay at tighter-spaced dose levels.

DETAILED REVIEW

Test Article:

Dylox Technical, a white crystalline solid, 97.3% ai, dissolved in distilled water for testing.

Procedures:

In a preliminary cytotoxicity test, cultures of CHO cells (CCL 61, from the American Type Culture Collection, Rockville, MD) were exposed to the solvent, DW, or to nine concentrations of test article ranging from 0.1 to 1000 ug/mL for 4 hours without activation, and 2 hours in the presence of proven S9 mix, following which the cells were washed free of treatment media and allowed to recover for 20 to 24 hours. Relative cell survivals and cell cycle delay (cytotoxicity of each treatment relative to solvent control) were used to select dose levels for the SCE assay.

For the main SCE assay, duplicate unactivated cultures were exposed to test article for a total of 26 to 32 hours; 2 hours after initiation of treatment, 0.01 mM bromodeoxyuridine (BrdUrd) was added, and Colcemid (0.1 ug/mL) for the last 2 hours. In the presence of S9 mix, cells were exposed to test article for only 2 hours, washed free of the treatment, then refed with fresh medium containing 0.01 mM BrdUrd for 24 to 30 hours, 0.1 ug/mL Colcemid being added for the final 2 hours.

Metaphase cells collected by the action of Colcemid were harvested by mitotic shake-off, pelleted by mild centrifugation (800 rpm for 5 minutes), suspended in 0.07 M KCl for 4 to 8 minutes, then fixed in Carnoy's Fluid (3 methanol:1 acetic acid), and stored therein overnight, or longer, until microscope slides were prepared.

In order to prepare slides for SCE counts, the fixed cells were drained to only a minimum of Carnoy's Fluid, resuspended in fixative, two drops of cell suspension applied onto a moist slide and allowed to air-dry overnight. The dried slides were stained with Hoechst 33258, mounted in phosphate buffer, exposed to uv light at 60 °C for 4 minutes, then counterstained with Giemsa (modified FPG technique).

Stained slides were coded using random numbers, and read "blind" with regard to treatment group. A total of four test article doses were scored, including the highest where sufficient second-division metaphases (M-2) were available. Twenty-five cells were scored for SCE/cell from each duplicate culture and treatment group means calculated (50 cells per treatment). In addition, percentages of first-division (M-1), second-division (M-2) and third-division (M-3) metaphases were recorded for each treatment flask.

To be considered positive, the author proposed that the test article should induce either: 1) a doubling of the SCE frequency over the solvent control level at three or more doses, or 2) a dose-response and statistically significant increase over three or more dose levels in the absence of doubling of the background. On the other hand, a statistically significant increase at only one or more dose levels, with no dose-response, is assessed as "equivocal," or as "negative," depending upon the magnitude of the response and the number of dose levels affected. Student's t-test was used to compare the mean SCE/cell of each treatment group with that of the solvent control.

To be considered a valid assay, the author proposed that the mean SCE/cell in the untreated (negative) control must be 16 or less, while that of positive controls must be significantly increased over the negative control.

Triethylenemelamine (TEM, 0.025 ug/mL) served as positive control for the nonactivated assay, and cyclophosphamide (CP, 2.5 ug/mL) was the positive control for the S9-activated study. The solvent vehicle (DW) served as solvent control, and growth medium as untreated control.

Results:

Based upon survival and cell cycle delay in the cytotoxicity tests, the author selected four dose levels for nonactivated SCE assays: 100, 50, 25, and 10 ug/mL; and four levels for activated SCE tests: 300, 150, 75, and 30 ug/mL (Tables 1 and 2 attached to this review). The criteria for selecting the high doses for the SCE assays are found in the Study Protocol appended to the Final Report (Appendix I). First, the high dose is selected from a level giving at least 50 percent toxicity (reduction in growth compared to solvent control), with a sufficient number of M-2 metaphase cells for evaluation (at least 25 percent). Three more lower doses are then chosen, covering at least a tenfold range. These criteria were satisfied as Tables 1 and 2 show. While relative cell growth was affected at 300 and 1000 ug/mL (decreased by 50 percent) in the nonactivated toxicity assay (Table 1), these levels were not considered useful because of severe cell cycle delay resulting in an absence of M-2 cells. Hence, the author selected the next level as the high dose, 100 ug/mL, where cell growth relative to control was minimally affected, and even though a 50 percent delay in cell cycle kinetics was produced, sufficient M-2 cells were available for analysis. The criteria and selection process were the same for the activated toxicity study. But in this case only the 1000 ug/mL dose was considered not to be useful, since cell growth decreased by 55 percent and no M-2 cells were available for analysis. The next lower dose provided a suitable toxic level for an HDT, namely, 73 percent cell growth, and 24 percent M-2 cells.

In nonactivated cultures treated for 26 to 32 hours, the author acknowledged that two assays were performed ("due to technical error"), but only the results of "the second assay are reported" (Table 3, attached to this review). Statistically elevated ($p < 0.01$) dose-responsive increases in SCEs were found at the two top doses, 100 and 50 ug/mL. Although the response at the next lower level, 25 ug/mL, was increased over solvent and untreated controls, the increase was apparently not significant. The background SCE level in untreated and solvent controls was 11 to 18 and 12 to 26, respectively, whereas the positive control, TEM, induced an average of 60.94 SCEs/cell, a highly significant value over the treated control ($p < 0.01$, Student's t-test). It should also be noted that except for the HDT, cell cycle kinetics were comparable to the solvent (DW) in percentage of available M-2 cells. The positive control (TEM) cultures also showed a moderately severe degree of cell cycle delay.

Two-hour treatment in the presence of activation resulted in statistically elevated increases in mean SCE/cell relative to DW control at three dose levels, 300 and 150 ug/mL ($p < 0.01$), and at 75 ug/mL ($p < 0.05$) (Report Table 4, attached). The positive control, CP, induced an average of 36.84 SCEs/cell, significantly elevated ($p < 0.01$) above the controls (11.31 and 11.68). In contrast to the responses in nonactivated cultures, cell cycle kinetics were equally distributed at all test levels, with no delay in production of M2 cells compared to controls. Secondly, the response at the three active test dosages is minimally dose-responsive (if at all). Finally, CP cultures showed no cell delay.

The author concluded that Dylox Technical is positive in the SCE assay in CHO cells, since it induced dose responsive and statistically significant increases in the frequency of SCEs both in the presence and absence of metabolic activation (Aroclor induced rat liver S9).

TB Evaluation:

The study appears to have been well conceived and carried out in a generally adequate manner. However, there were certain considerations, both in the conception and performance, which compromise the results generated, and thereby the conclusions postulated.

1. First of all, it is evident that test chemical effects on cell growth and cell kinetics can vary, as shown by the large discrepancies between toxicity tests and main SCE assays. Solution: Do repeat cytotoxicity assays at more finely tuned dose schedules, to evolve high doses more closely "maximally tolerated doses."

2. This above reservation is generated from the lack of data in the results of the SCE assays, namely, cell growth.

3. The results for the nonactivated assay did not fully satisfy the author's own criteria for a positive, since neither a doubling over solvent control at a minimum of three dose levels, nor statistically significant increases (t-testing) over a minimum of three dose levels, was observed. Since there is evidence for a dose-response, however, the assessment would have to be stronger than "equivocal" (certainly not "negative").

4. Results of the activated assay were even more difficult to interpret. The response at the three highest doses presents a very flat "dose-response" (if it can be so defined at all), and no evidence of toxicity (reduced cell growth) data, which would have been useful if provided; in addition there was normal cell cycle kinetics, i.e., plenty of M-2 cells.

5. These uncertainties in interpreting this study would be resolved by more data on cytotoxicity, plus a completely independent confirmatory experiment, or at least additional data already available, as suggested in the report that the nonactivated assay was repeated once ("due to technical error").

Therefore, our assessment of this study is ACCEPTABLE in the absence of activation, exhibiting positive results for the induction of SCE at 50 and 100 ug/mL, with evidence for a dose response. In the presence of S9 activation, on the other hand, we judge this portion INCONCLUSIVE, providing presumptively positive induction of SCE at 75, 150, and 300 ug/mL, with minimal evidence for a dose response, thus requiring a confirmatory assay at tighter-spaced dose levels.

Attachments

006745

Pages 40-43 - *Access to FIFRA health and safety data is restricted under FIFRA section 10(g)*

006745

Primary Reviewer: William B. Greear, M.P.H. 3/23/89
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: Albin S. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch (TS-769C)

ABK 3/23/89

DATA EVALUATION REPORT

Study Types: Subchronic Neurotoxicity: 90-Day Study

TOX Chem No.: 385

MRID No.: 403512-00

Accession No.: N/A

Test Material: Trichlorofon Technical

Synonyms: Dylox

Study No.: Mobay Report No. 94821; also Nos. 86-418-03 and 86-498-02

Sponsor: Mobay Corporation
Kansas City, MO

Testing Facility: Mobay Corporation
Health, Environment and Safety
Corporate Toxicology Department
Stilwell, KS 66085-9104

Title of Report: Subchronic Delayed Neurotoxicity Study of Trichlorofon Technical (DYLOX®) with Hens

Authors: R.H. Hayes and W.W. Ramm

Report Issued: August 28, 1987

Conclusions:

Does not produce "delayed neurotoxicity."

NOEL = 9 mg/kg
LEL = 18 mg/kg (based on slight axonal degeneration)

Classification: Core-Guideline (tentative - see discussion)

A. Materials:

1. Test Compound - Trichlorofon technical; Description: white powder; Batch No.: not provided; Purity: 98.8%; Contaminants: not provided.

   Positive Control - Tri-ortho-tolyl-phosphate (TOCP); Description: colorless liquid; Batch No.: P 1517; Lot No. A 13 B; Purity: 99%; Contaminants: not provided.

2. Test Animals - Species: White Leghorn Hens (Gallus gallus); Age: 12 months; Weight: 1.0 to 1.4 kg; Source: Colonial Poultry Farm, Pleasant Hill, Missouri.

B. Study Design:

A dose-range finding study was conducted using dose levels of 0, 9, 18, 24, and 27 mg/kg administered to 5 hens/group over a 24-day period. One animal died in each of the 18 and 24 mg/kg groups. Two hens died in the 27 mg/kg group. Whole blood cholinesterase (ChE) activity was decreased 20, 36, and 36 percent in the 9, 18, and 24 mg/kg groups. Whole blood ChE activity was not measured in the group that received 27 mg/kg due to mortality (no blood samples were taken). The dose levels selected for testing were 3, 9, and 18 mg/kg.

1. Animal Assignments - Animals were assigned to the following test groups by body weight using computer randomization programs.

Test Group	Dose (mg/kg)***	Main Study 13 Weeks No Females	Interim Sac.
Negative Control #1** (concurrent control)	0	12	
Negative Control #2* (concurrent study control)		12	
Low Dose	3	12	
Mid Dose	9	12	
High Dose	18	12	
Postive Control (TOCP)	45	12	

*This group was derived from a concurrent study, No. 86-498-02.
**This group represents the concurrent controls for this study, No. 86-418-03.
***Administered five times per week.

Upon receipt, the animals were acclimated to laboratory conditions for a period of 13 days. The hens were housed in stainless steel cages with wire mesh floors. Pelletized wood bedding was used in litter pans, which were changed at least twice weekly. Cages were washed every 3 weeks. Temperature and relative humidity were maintained at 16 to 27° C and 45 to 75 percent, respectively. A 12-hour on/12-hour off light cycle was maintained.

2. Dose Preparation - The test material was dissolved with acetone and mixed with corn oil (corn oil:acetone; 92:8 - first 5 days, 90:10 - thereafter) for gavage at 1 mL/kg body weight daily. Dosing solutions were prepared at 5-day intervals (weekly) and stored in a refrigerator prior to administration. TOCP was administered in 100% corn oil at a rate of 1 mL/kg body weight. The two vehicle control groups received equivalent amounts of corn oil:acetone or corn oil at 1 mL/kg body weight. The hens were weighed weekly and their doses calculated for each week according to these weights.

   Results - It was stated that analysis of the active ingredients by the sponsor indicated the test article was stable during the study. The test material was stable in the corn oil dosing solutions for 7 days while refrigerated. The mean concentrations of the test material in corn oil were 90 to 100 percent of the nominal dose.

3. Animals received Ralston Purina's Layena Poultry Feed 6501 and water ad libitum.

4. Statistics - Body weight, feed consumption and ChE activity were subjected to Analysis of Variance (ANOVA) followed by Duncan's Multiple Range test if the ANOVA F-test showed significance at $p \leq 0.05$. Statistically significant differences were reported at the 95 percent confidence level.

5. Quality assurance was conducted from September 3, 1986 to August 5, 1987. The statement was signed by E.J. Hixson on August 28, 1987.

C. Methods and Results:

1. Observations - Animals were observed for signs of toxicity and mortality twice daily except on holidays and four weekends when observations were made once daily. In addition, the hens were observed weekly while being subjected to a ladder-climbing test. The ladder was composed of seven rungs made of 1-inch wood dowels, wrapped with abrasive tape and placed 5 inches apart.

The bottom rung was 19 inches above the bottom platform and the top platform was 24 inches above the bottom platform. Each test consisted of three 2-minute trials. Motor coordination, locomotor ataxia and paresis were qualitatively appraised by forcing the hens to move on a 3.2 m^2 turfed area, once a week for approximately 2 to 5 minutes.

Results - One hen in each of the 9 and 18 mg/kg groups were sacrificed in extremis at the end of the fourth week due to infection. Several hens in the positive control group died prior to termination beginning in week 4. As a result, four hens in the control groups were sacrificed after 2 months in order to compare their tissues with those in the positive control group. No mortality could be attributed to administration of the test material. There were no significant differences in appearance and behavior of hens in the two control groups and hens in the 3 and 9 mg/kg groups. However, during the first 4 weeks on test it was stated that hens in the 18 mg/kg group exhibited signs of ataxia and decreased activity. During week 5 through 9, the incidence of these signs decreased. During the last 4 weeks the signs intensified. In addition, salivation was observed within the first few hours of treatment. It is stated that in most hens ataxia and decreased activity occurred shortly after treatment and not just prior to treatment indicating that the findings were related to repetitive acute intoxication rather than to delayed neurotoxicity. Hens in the positive control group exhibited persistent ataxia and decreased activity from week 4 until termination. During forced motor activity the majority of hens in the two control groups and hens in the 3, 9, and 18 mg/kg groups appeared to be quite similar. One hen in the 9 mg/kg group and one hen in the 18 mg/kg group had signs of ataxia during weeks 4 and 3, respectively. The hen in the 18 mg/kg group recovered. The hen in the 9 mg/kg group was sacrificed in extremis. Death was attributed to a systemic infection. Hens in the TOCP group exhibited ataxia, knee-bending and decreased activity during week 4. These signs increased in severity over the remainder of the test period. [There is a discrepancy in the report of the Appendix IV Group Turf Test Observations on page 48. During weeks 1 and 2 there is mention of Hen No. 9 and during week 8 Hen No. 5 is mentioned. Hen numbers for this group (18 mg/kg) only have numbers ranging from 3051 to 3062. This discrepancy requires resolution.] The results of the forced ladder climbing test revealed that there were no significant

differences between the control groups and the test groups. By the end of the third week all hens in the TOCP group had difficulties climbing the ladder and used their wings for balance. During the last 5 weeks of the study the surviving hens in the TOCP group were unable to sit on the ladder.

2. Body Weight - The hens were weighed weekly.

Results - Mean body weights of the hens in the control #1 group were 1.1 to 1.2 kg. During weeks 6, 8, 13, and 14 the body weights of hens in the 3 mg/kg group were significantly increased when compared to the control #1 group. During week 14 the body weight of hens in the 9 mg/kg group were significantly increased when compared to the control #1 group. The body weights of hens in the 18 mg/kg group were significantly decreased at 2, 3, 5, 9, 12, and 13 weeks when compared to the control #2 hens. The mean group body weights of hens in the test groups ranged from 1.0 to 1.2 kg. The body weights of hens in the TOCP group were significantly decreased at weeks 4 to 14 when compared to control #2 hens. The mean group body weights of hens in the TOCP group ranged from 0.7 to 1.2 kg.

3. Food Consumption and Compound Intake - Food consumption was determined on a weekly basis.

Results - Food consumption was slightly increased in hens in the 3 and 9 mg/kg groups. Food consumption of hens in the 3 mg/kg group was significantly increased during week 2 when compared to control #2 hens. Food consumption was slightly decreased in hens in the 18 mg/kg group. Significant decreases in food consumption occurred during weeks 2 and 5. During weeks 4, 5, and 6, hens in the TOCP group showed significant decreases in food consumption when compared to control #2 hens. During weeks 10, 11, and 12, hens in the TOCP group exhibited significant increases in food consumption when compared to control #2 hens; however, the increase was attributed to spillage.

4. Whole Blood ChE Activity - Determinations were made prior to study initiation and during weeks 1, 2, 6, 9, and at termination. Blood samples were taken from the wing vein.

Results - ChE activity was significantly decreased in the 9 and 18 mg/kg group during weeks 2, 6, 9, and 14. Significant decreases in ChE activity were also observed in the 3 mg/kg group during weeks 9 and 14; however, a 20 percent decrease or greater was observed only during

week 14. Hens in the TOCP group exhibited significant decreases in ChE activity during weeks 2, 9, and 14; however, a 20 percent decrease or greater was observed only during weeks 2 and 14. The analysis during week 14 included only one surviving hen.

5. Sacrifice and Pathology - All hens were necropsied. This included those hens found dead, those sacrificed in a moribund condition, and those sacrificed at termination. The CHECKED (X) tissues were collected for histological examination.

X		X	
	Neurologic		Neurologic
X	Brain	X	Lumbosacral spinal cord
X	Cervical spinal cord (upper and lower)	X	Sciatic nerve (right and left)
X	Thoracic spinal cord	X	Tibial nerve (right and left)
		X	Peroneal nerve (right and left)
			Other
		X	Gross lesions

Results

a. Gross Pathology - Four hens in the 18 mg/kg group had enlarged, thickened esophagi. One hen also had a thickened crop and proventriculus. It was stated that the increased thickness was limited to the muscular and submucosal tissues. One hen in the 18 mg/kg group was sacrified in extremis due to egg yolk peritonitis. Mild egg yolk peritonitis was also seen in one hen in the 9 mg/kg group and in one hen in the 3 mg/kg group. One hen in the 9 mg/kg group was sacrificed in extremis due to a systemic infection. Eight of 12 hens in the TOCP group were sacrificed in extremis; two were found dead and two were sacrificed at termination. Several of the hens were emaciated.

b. Microscopic Pathology - The thickened esophagus, crop and/or proventriculus seen in four hens in the 18 mg/kg group was confirmed microscopically. The lesion was characterized by prominent hypertrophy with lesser hyperplasia of the muscularis mucosa and muscle layers of the tissues. The incidence of neurological lesions were similar among the control and test groups with the exception of axonal degeneration of

the brain and/or spinal cord. The incidence of axonal degeneration is provided in the table below:

Axonal Degeneration of the Brain and/or Spinal Cord in Hens

	Dose Level (mg/kg)					
Lesion	Concurrent Control #1	Concurrent Study Control #2	3	9	18	TOCP at 45 mg/kg
Axonal degeneration	5/11(45%)	2/11(18%)	5/11(45%)	3/11(27%)	9/11(82%)	12/12(100%)

The data indicate that there was an increase in the incidence of axonal degeneration in hens in the 18 mg/kg group when compared to controls. The severity of the lesion in the control and test hens was minimal (Grade 1) with the exception of one hen in the 18 mg/kg group where the lesion was mild (Grade 2). Hens treated with TOCP had prominent axonal degeneration with large, eosinophilic swollen and often fragmented axons, with increased severity of degeneration of digestion chambers and macrophage accumulation when compared to controls.

D. Discussion:

One hen in each of the 9 and 18 mg/kg groups were sacrificed in extremis due to infection. Several hens in the TOCP group died or were sacrificed in a moribund condition due to the toxicity of the compound administered. There were no significant differences in appearance and behavior of hens in the control and 3 and 9 mg/kg groups. Hens in the 18 mg/kg groups exhibited signs of ataxia and decreased activity shortly after dosing. Later in the day these signs disappeared. In addition, salivation occurred in the latter few weeks of dosing indicating that the signs could be attributed to the acute toxic effects produced by the test material. Hens in the control and test groups were similar in performace of the forced motor activity test and the forced ladder climbing test. Body weight and food consumption were slightly increased in the 3 and 9 mg/kg groups (on occasion) when compared to controls. Body weights and food consumption of hens in the 18 mg/kg group were significantly decreased. Whole blood ChE activity was significantly decreased in hens in the test groups. Four hens in the 18 mg/kg group had thickened esophagi and/or crop and proventriculus.

Histological examination of hens in the TOCP group revealed lesions characteristic of delayed neurotoxicity. Hens in the 18 mg/kg group had an increase in the incidence of axonal degeneration when compared to controls. The severity of the lesion was minimal to mild. During the observation period no overt signs of delayed neurotoxicity were observed in the 18 mg/kg group.

Conclusions:

The highest dose tested (18 mg/kg) is a maximum tolerated dose based on the results of the range-finding study and the degree of acute toxicity observed in hens in the 18 mg/kg group. There was no overt indications of a response characteristic of classical "delayed neurotoxicity"; however, histologically a slight effect on nervous tissue manifested as axonal degeneration was present in hens in the 18 mg/kg groups. The NOEL is 9 mg/kg and the LEL is 18 mg/kg based on slight axonal degeneration.

Classification:

Core-Guideline (tentatively, based on the response to TB's questions).

[The sponsor must explain the discrepancy in reporting results for the forced motor activity (Turf) test in which results were provided for Hens Nos. 5 and 9, when the hens were given 4-digit numbers.]

Stanley B. Gross 5/28/87 006665

006745

Reviewed by: Stanley B. Gross, Ph.D.
Section 7, Toxicology Branch (TS-769C)
Secondary reviewer: Albin B. Kocialski, Ph.D.
Section 7, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Rat Metabolism (Guideline 85-1)

Accession No: MRID # 404381-01.

Test Material: Trichlorfon

Synonyms: Dylox, Dypterix, Neguvon.

Testing Facility: Mobay Corporation, Agricultural Chemicals Division, Stilwell, KS 66085.

Authors: H.S. Shaw II, R.B. Minor, P.L. Freeseman, L. Pfanche and V.J. Lemke.

Report Issued: Excretion and Metabolism of Dylox in Rats. Mobay Report No. 94594, June 30, 1987.

SUMMARY

Trichlorfon labeled in the 2C position of the trichlorohydroxy moiety was administered to rats under four regimens: 1) low dose (0.2 mg/kg) by gavage; 2) low dose (0.2 mg/kg) by gavage after 2 weeks of unlabeled administration; 3) low dose (0.2 mg/kg) given by intravenous injection and 4) high dose (20 mg/kg) by gavage.

Eighty to 89% of the radioactivity was excreted in the first 24 hour periods, with 40 to 50% in the urine, 20% in the feces and 20% in the expired air. One to 2% of the radioactivity was found in the tissues after 96 hours. The radioactivity in the urine and feces each indicated 7 major chromatographic peaks. None of the peaks were adequately characterized as specific metabolites and appeared to be different chemicals based on different retention times.

The investigators postulated that trichlorfon was converted to DDVP and its metabolites, however, neither DDVP or any DDVP metabolites were demonstrated to occur.

Conclusions. The study is considered SUPPLEMENTARY. The data for the kinetics of excretion and deposition of trichlorfon are acceptable however, the identification of metabolites is inadequate. Discussions from the literature raise questions concerning the conversion of TCF to DDVP invivo. This study did not demonstrate this conversion.

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MATERIALS AND METHODS

A copy of the Materials and Methods taken from the Report is included here as Appendix 1. In summary:

1. Test Materials.

Radiolabeled TCF. 14C radiolabeled trichlorfon (*TCF) labeled in the 1-hydroxyethyl carbon was synthesized by the method of Poje (cited in the report). The specific activity of *TCF was determined to by 13.74 mCi/mmole (718,614 dps/0.2 mg trichlorfon). The purity of *TCF was 99+% established by thin layer chromatography (TLC) and high-pressure liquid chromatography (HPLC). Unlabeled TCF was determined to be 96+% pure by HPLC.

2. Test animals:

Species: Rats.
Strain: Sprague Dawley .
Age: Not provided.
Weights: Not provided.
Source: Sasco Inc., St. Louis, MS.

3) Experimental Design

Four experiments were carried out using 5 animals/sex/group:

LDE (Low dose experiment): single gavage dose of *TCF in water at 0.2 mg/kg.
LDCE (Low dose "chronic" experiment): 10 single gavage doses using TCF in water at 0.2 mg/kg followed by a single gavage dose at 0.2 mg/kg of *TCF in water.
LDIE (Low dose intravenous experiment): single 0.2 mg/kg of *TCF in water given IV via tail vein.
HDE (High Dose Experiment): single gavage dose of *TCF 20 mg/kg in water by gavage.

A repeat HDE "Mini-Study" was also carried out using only two animals (one male and one female) and only evaluating the metabolites found in the feces.

The radioacitivy was measured in urine, feces, expired air during days 1, 2, 3 and 4 and in tissues 4 days after the adiministration of *TCF. The radioactivity was measured in individual samples of urine, feces and exhaled air collections. Metabolites found in urine, feces and tissues were obtained from pooled samples/organs.

4) Animal Care and Caging .

The rats were housed in glass metabolism chambers which allowed separate collection of urine and feces and expired gases. Expired radiolabeled 14C was collected in a CO2 absorption tower containing NaOH.

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5) Radioassay

Urine. Aliquots of urine from each collection was assayed in triplicate for radioactivity by liquid scintillation using a Beckman LS 9000 Liquid Scintillation Counter (LSC).

Feces, Tissues and Blood . Tissues from 5 animals (5/sex/experimental group) were pooled for analyses. These samples were [illegible] 457 [illegible] Acetonitrile LLE [illegible] Acetonitrile LL was then analyzed for radioactivity using LSC. The tissues assayed are listed in Table IV from the report which is discussed under Results.

Expired Air. Composites from the NaOH trap samples were prepared by combining 5 ml aliquots from five individual traps from each male or female for each 24 hour interval. The 14CO2 from each of these composites were processed through a series of extraction steps and radioassayed and tested thin layer chromatography (TLC) with reverse to check the 14CO2 purity.

Metabolites. The radioactivity of solutions and tissue extracts were measured using LSC. Individual urinary metabolites were separated using high pressure liquid chromatography (HPLC) and quantitated using a lithium glass scintillation cell which was approximately 59% efficient for 14C. The limit of detection of the lithium cell was determined to be equivalent to 1000 dpm.

6) Metabolite Assays.

Metabolite Identification. Thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC) were used to characterized the following standards as possible metabolites:

TCF	Trichlorfon
DesTCF	Desmethyl TCF
TCE	Trichloroethanol
TCA	Trichloroacetic acid
CH	Chloral hydcate
DDVP	Dichlorvos
DesDDVP	Desmethyl dichlorvos
DCE	DichoroethAnol
DCA	Dichloracetic acid
DCald	Dichloraldehye

The structures for these compounds are shown in Figure 1 taken from page 31 of the report. The purity of the standards was established using HPLC and mass spectometry. Most were above 90% pure. The mobility characteristics in TLC and HPLC analyses were also established.

Urinary Metabolites. Acid Hydrolysis. Since most (approximately 90%) of the radioactivity from the treated animals was excreted within 24 hours after dosing, the first 24 hour urine collections urine collections were pooled and analyzed for TCF metabolites. Aliquots (2 ml) from these pooled urine samples were hydrolyzed with HCl, neutralized and extracted into ether. The extract was then concentrated, radioassayed and subjected to TLC.

Enzyme Hydrolysis-- Sulfatase/ Beta Glucuronidase. Samples of pooled rat urine from HDE was adjusted to pH 5 and incubated with sulfatase derived from abalone entrails and eventually subjected to HPLC separation. Other samples of pooled urine from the HDE were also incubated with beta glucuronidase and the resulting metabolites also subjected to HPLC separation.

Feces. One day female composite feces sample from the HDE were combined with Hyflo-Super Cel and filtered. The filtrate was subjected to HPLC analyses.

RESULTS

1) Trichlorfon Residues.

Tables II and III (pages 25 and 26 from the report) show the distribution of the radioactivity during the first 24 hours (Table II) and after 96 hours (Table III). The recovery of the administered radioactivity after 96 hours was quite high (approximately 100%) for all 4 experiments and most (80% to 90%) of the label was found in the excreted in the urine (40-50%), feces (approximately 20%) and expired air (approximately 20%). One to 2% of the radioactivity was found in the tissues after 96 hours.

Table IV (taken from page 27 of the report) lists the radioactivity found by the individual tissues. The residues are expressed as ppm of Dylox residues rather than percent of adminisited dose. In all of the experiments the most of the tissues from the female rats exceed those of the male rats. The residues from the LDE were comparable to the loading dose experiment (LDCE). Based on the HDE study, liver, kidney, bone and gonads, respectively had the higher tissue concentrations.

2) Trichlorfon Metabolites in Urine.

Figure 7 (taken from page 37 of the report) represents a typical HPLC chromatogram of urine from the HDE experiment (curve A) and the same chromatogram spiked with DCA standard. Peaks U1 to U7 were not identified and U4 was different from the DCA peak. HPLC peaks from acid treated urine were shown for male and females of the LDE, LDIE and LDCE experiments and again, the individual peaks are not identified. It should be noted that the investigators had standards for 9 possible metabolites (shown in Table 1, from page 24 of the report) which could have been used to help identify

the individual peaks from the HPLC chromatograms. This was also the situation in for the chromatograms of the enzyme hydrosis of urine (such as Figure 10) and the chromatographs for the fecal metabolites (Figure 17).

DISCUSSION

1) Kinetics of Residue Distribution.

The kinetics based on the radiolable analyses appeared to be acceptable and consistant with the studies of other investigator (WHO, 1971; CEC, 1977; Hayes, 1982).

2) Metabolites.

The characterization of the metabolites was completely unsatisfactory. The investigators identified 10 metabolites (Figure 1) which might be formed by the biotransformation of TCF and/or DDVP and adequately characterized each of these on for each of the analytical methods used. In the metabolite identification, however, the investigators only attempted to characterize one of the peaks (DCA) in each of the chromatographic separations. This peak did compare apropriately to a DCA peak based on the release times determined with pure materials. The investigators failed to characterize any of the other 4 to 7 peaks found in urine and feces. Thus the characterization of the metabolites was completely inadequate.

3) Lack of DDVP/Metabolites.

The authors assumed that TCF formed DDVP and other metabolites as shown in their Figure 19. However, they did not identify DDVP as such nor any of the metabolites which might be expected to be formed from DDVP. Bull and Ridgeway (1969) discussed the discrepancy between studies, some reporting the conversion of TCF to DDVP and others not. They noted the non-biological conversion TCF to DDVP reported by Bathel et al. (1955) under acidic and alkaline conditions in vitro. The Commission of European Communities (CEC, 1977) discusses data that indicates transformation occurs in mammals, however, only to a small extent. This issue will need further analysis, especially a review of the different studies. Of concern is the possibility that DDVP/metabolites may form during the analysis of metabolites as an artifact.

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Pages 58-73 - *Access to FIFRA health and safety data is restricted under FIFRA section 10(g)*